some unexpected shape transitions undergone by lipid vesicles generated from membrane-protein interactions. Based on our quantitative model and findings, we highlight the physical principles that unify CME in apparently distinct yeast and mammalian cells.

1577-Pos Board B307
Dynamics of Intracellular Clathrin Carriers
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Clathrin-based carriers are responsible for a large fraction of the membrane traffic between the plasma membrane, endosomes and the trans-Golgi network (TGN). Clathrin/AP1- and clathrin/AP3-coated membrane carriers originate from endosomes and TGN. Efforts to follow these intracellular carriers had not been successful because the rapid three-dimensional movement of endosomes has not permitted one to distinguish initiation of assembly or dissociation of their coat from passage into or out of the imaging plane. Here, we report the real-time visualization of these structures in living cells reliably tracked by rapid, three-dimensional imaging with the use of a spinning-disk confocal microscope. The dynamic characteristics of the intracellular clathrin/AP1 and clathrin/AP3 carriers are similar to those of endocytic clathrin coated pits and vesicles. We found no evidence supporting association of dynamin2 with clathrin/AP1 or clathrin/AP3 carriers at any stage during their lifetime. This observation suggests that an alternative budding mechanism, yet to be discovered, is responsible for the scission step of clathrin/AP1 and clathrin/AP3 carriers.

1578-Pos Board B308
Real-Time Endocytosis Imaging as a Rapid Assay of Ligand-GPCR Binding in Single Cells
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Most G protein- coupled receptors (GPCRs) do not generate membrane currents in response to ligand-receptor binding (LRB). Here, we describe a novel technique using endocytosis as a bioassay that can detect activation of a GPCR in a way analogous to patch-clamp recording of an ion channel in a living cell. The confocal imaging technique, termed FM endocytosis imaging (FEI), can record ligand-GPCR binding with high temporal (second) and spatial (micrometer) resolution. LRB leads to internalization of an endocytic vesicle, which can be labeled by a styryl FM dye and visualized as a fluorescent spot. Distinct from the green fluorescence protein-labeling method, FEI can detect LRB endocytosis mediated by essentially any receptors (GPCRs or receptors of tyrosine kinase) in a native cell/cell line. Three modified versions of FEI permit promising applications in functional GPCR studies and drug screening in living cells: 1) LRB can be recorded in ''real time'' (time scale of seconds); 2) internalized vesicles mediated by different GPCRs can be discriminated by different colors; and 3) a high throughput method can screen ligands of a specific GPCR.
Supported by grants from NSFC, MOST and the PKU-THU-Center for Life Sciences

1579-Pos Board B309
Role of Internalization of Cell Surface Receptors in Regulation of Cell Polarity
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Internalization of cell surface receptors is often stimulated after ligand binding. It plays important roles in biological processes such as signal desensitization, substance uptake, and signal transduction. Antagonist-stimulated internalization may be also important for cell polarization [1]. We have developed a general model to study the role of receptor internalization in the establishment of cell polarity. Our results show that regulated antagonist-stimulated internalization can provide an important mechanism to initiate cell polarization. It also provides additional regulation of signal amplification and noise filtering, which are important for the sensitivity and the accuracy of signal sensing. We discuss applications of our model to the study of yeast polarity and compare computational results with experimental observations.
[1] Polarization of the Yeast Pheromone Receptor Requires Its Internalization but Not Actin-dependent, D.V. Suchkov et al, Mol Biol Cell. 2010 May 15; 21(10): 1737-1752.

1580-Pos Board B310
Molecular Mechanisms of Endocytosis and Exocytosis in Yeast Studied by High-Resolution Membrane Capacitance Measurements
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Exo- and endocytosis are crucial in numerous physiological processes in eukaryotic cells, such as polar growth, cell division, motility or signaling. Endo- as well as exocytotic events result in small changes of the cell surface area, which can be resolved in electrophysiological recordings in the form of discrete steps in membrane capacitance. By applying high resolution recordings with yeast protoplasts, we were able to resolve discrete changes in membrane capacitance mainly in the range 0.2-1.2fF, corresponding to fusion and fission of single vesicles of 80-250nm. Analysis of exo- and endocytosis in the yeast *sec6-4* mutant, which is characterized by a temperature dependent block of exocytosis, revealed low fission/fusion activity at room temperature. Incubation at the restrictive temperature of 37°C for 3h and shifting to the permissive temperature of 25°C resulted in a transient increase in endo-/exocytotic activity of up to 10-fold. This study demonstrates that patch clamp capacitance measurements in yeast provide a novel *in vivo* tool to examine the molecular mechanisms of endo-/exocytosis in this model organism. The recordings gain information in real time on the geometry of endo-/exocytotic vesicles, on the kinetics of the events and their dependence on signaling cascades and key proteins.

1581-Pos Board B311
Cellular Scale Biophysical Models of Membrane Sculpting by the Proteins During Endocytosis and Exocytosis
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We employ coarse-grained molecular dynamics (CGMD) simulations and continuum mechanics methods to quantify the membrane-bending action of exo70. CGMD simulations show that exo70 produces the strongest curvature when bound to a key lipid PIP2, and when dimerized. To relate these simulations to experiments, we first quantify the deformation field generated by exo70 using CGMD simulation, and then investigate the resulting cellular morphologies using a continuum mechanics model for membrane bending. In the first step, we characterize the deformation field by directly analyzing curved membrane conformations generated by CGMD simulations of mutant and wild-type exo70 on mixed lipid bilayers. Monte Carlo simulations yield membrane morphologies which we relate to cryo-transmission electron microscopy images of exo70 remodeling a GUV. This model agrees with experiments which show that PIP2 binding and dimerization are necessary for generating membrane tubulues and protrusions. We show that this multi-scale model allows us to relate the structural as well as thermodynamic details of exo70 membrane-binding and self-association to the induced deformation field and resulting cellular morphologies. We also extend our studies to ENTH, BAR, and ESCRT family proteins. We show that thermal undulations in the membrane and cooperativity in the curvature fields, collectively drive the membrane into different morphological states (buds, tubules, etc.) that resemble those in cellular experiments in vivo and vesicle experiments in vitro. We determine the relative stability of the above mentioned shapes based on the free energy of these membrane configurations, determined using the methods of Thermodynamic Integration (TI) and Bennett Acceptance (BA), and Widom Insertion techniques. Results are shown for the case of Exo70 protein and ENTH, and N-BAR domains and also compared against measurements determined from experiments.

1582-Pos Board B312
Real-Time Measurements of Exocytosis and Endocytosis in C. Elegans Neurons
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The nematode C. elegans is a powerful genetic system for studying the molecular basis of synaptic transmission. However, current approaches to analyze exocytosis and endocytosis in C. elegans are limited. We rely on assays that are either indirect (e.g., postsynaptic recordings from neuromuscular junctions) or with low time resolution (e.g., FM dye uptake and synaptic protein abundance). Here, we aim to develop the real-time analysis of neuronal exo- and endocytosis in C. elegans. We directly monitor membrane insertion and retrieval by following changes in the membrane capacitance (Cm). We